

(1) GENERAL INFORMATION

- (i) APPLICANT: LIN, LEU-FEN; COLLINS, FRANKLIN D.;
DOHERTY, DANIEL H.; LILE, JACK; BEKTESH,
SUSAN
- (ii) TITLE OF INVENTION: Glial Derived Neurotrophic Factor
- (iii) NUMBER OF SEQUENCES: 24
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Swanson & Bratschun, L.L.C.
 - (B) STREET: 8400 E. Prentice Avenue, Suite 200
 - (C) CITY: Englewood
 - (D) STATE: Colorado
 - (E) COUNTRY: USA
 - (F) ZIP: 80111
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 MB storage
 - (B) COMPUTER: IBM compatible
 - (C) OPERATING SYSTEM: MS DOS
 - (D) SOFTWARE: Wordperfect 5.1
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/182,183
 - (B) FILING DATE: 23-MAY-1994
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 07/788,423
 - (B) FILING DATE: 06-NOV-1991
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 07/774,109
 - (B) FILING DATE: 08-OCT-1991
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 07/764,685
 - (B) FILING DATE: 20-SEP-1991
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 07/855,413
 - (B) FILING DATE: 19-MARCH-1992
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Barry J. Swanson
 - (B) REGISTRATION NUMBER: 33,215
 - (C) REFERENCE/DOCKET NUMBER: SYNE-225C4
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (303) 793-3333
 - (B) TELEFAX: (303) 793-3433

(2) INFORMATION FOR SEQ ID NO:1

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (v) FRAGMENT TYPE: N-terminal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ser Pro Asp Lys Gln Ala Ala Ala Leu Pro Arg Arg Glu Arg Asn Xaa

Gln Ala Ala Ala Ala Ser Pro Asp Asn
20 25

- (2) INFORMATION FOR SEQ ID NO:2
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear
(v) FRAGMENT TYPE: internal fragment
(ix) FEATURE: Xaa is either Lys or Gln
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Xaa Ile Leu Lys Asn Leu Gly Arg Val Arg Arg Leu
5 10

- (2) INFORMATION FOR SEQ ID NO:3
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 900 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: nucleic acid for rat GDNF
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCCCCGGGCT GCAGGAATTC GGGG GTC TAC GGA GAC CGG ATC CGA GGT 48
Val Tyr Gly Asp Arg Ile Arg Gly
-90

GCC GCC GCC GGA CGG GAC TCT AAG ATG AAG TTA TGG GAT GTC GTG 93
Ala Ala Ala Gly Arg Asp Ser Lys Met Lys Leu Trp Asp Val Val
-85 -80 -75

GCT GTC TGC CTG GTG TTG CTG CAC ACC GCG TCT GCC TTC CCG CTG 138
Ala Val Cys Leu Val Leu Leu His Thr Ala Ser Ala Phe Pro Leu
-70 -65 -60

CCC GCC GGT AAG AGG CTT CTC GAA GCG CCC GCC GAA GAC CAC TCC 183
Pro Ala Gly Lys Arg Leu Leu Glu Ala Pro Ala Glu Asp His Ser
-55 -50 -45

CTC GGC CAC CGC CGC GTG CCC TTC GCG CTG ACC AGT GAC TCC AAT 228
Leu Gly His Arg Arg Val Pro Phe Ala Leu Thr Ser Asp Ser Asn
-40 -35 -30

ATG CCC GAA GAT TAT CCT GAC CAG TTT GAT GAC GTC ATG GAT TTT 273
Met Pro Glu Asp Tyr Pro Asp Gln Phe Asp Asp Val Met Asp Phe
-25 -20 -15

ATT CAA GCC ACC ATC AAA AGA CTG AAA AGG TCA CCA GAT AAA CAA 318

Ile	Gln	Ala	Thr	Ile	Lys	Arg	Leu	Lys	Arg	Ser	Pro	Asp	Lys	Gln		
-10					-5					1				5		
GCG	GCG	GCA	CTT	CCT	CGA	AGA	GAG	AGG	AAC	CGG	CAA	GCT	GCA	GCT		363
Ala	Ala	Ala	Leu	Pro	Arg	Arg	Glu	Arg	Asn	Arg	Gln	Ala	Ala	Ala		
				10					15					20		
GCC	AGC	CCA	GAG	AAT	TCC	AGA	GGG	AAA	GGT	CGC	AGA	GGC	CAG	AGG		408
Ala	Ser	Pro	Glu	Asn	Ser	Arg	Gly	Lys	Gly	Arg	Arg	Gly	Gln	Arg		
				25					30					35		
GGC	AAA	AAT	CGG	GGG	TGC	GTC	TTA	ACT	GCA	ATA	CAC	TTA	AAT	GTC		453
Gly	Lys	Asn	Arg	Gly	Cys	Val	Leu	Thr	Ala	Ile	His	Leu	Asn	Val		
				40					45					50		
ACT	GAC	TTG	GGT	TTG	GGC	TAC	GAA	ACC	AAG	GAG	GAA	CTG	ATC	TTT		498
Thr	Asp	Leu	Gly	Leu	Gly	Tyr	Glu	Thr	Lys	Glu	Glu	Leu	Ile	Phe		
				55					60					65		
CGA	TAT	TGT	AGC	GGT	TCC	TGT	GAA	GCG	GCC	GAG	ACA	ATG	TAC	GAC		543
Arg	Tyr	Cys	Ser	Gly	Ser	Cys	Glu	Ala	Ala	Glu	Thr	Met	Tyr	Asp		
				70					75					80		
AAA	ATA	CTA	AAA	AAT	CTG	TCT	CGA	AGT	AGA	AGG	CTA	ACA	AGT	GAC		588
Lys	Ile	Leu	Lys	Asn	Leu	Ser	Arg	Ser	Arg	Arg	Leu	Thr	Ser	Asp		
				85					90					95		
AAG	GTA	GGC	CAG	GCA	TGT	TGC	AGG	CCG	GTC	GCC	TTC	GAC	GAC	GAC		633
Lys	Val	Gly	Gln	Ala	Cys	Cys	Arg	Pro	Val	Ala	Phe	Asp	Asp	Asp		
				100					105					110		
CTG	TCG	TTT	TTA	GAC	GAC	AGC	CTG	GTT	TAC	CAT	ATC	CTA	AGA	AAG		678
Leu	Ser	Phe	Leu	Asp	Asp	Ser	Leu	Val	Tyr	His	Ile	Leu	Arg	Lys		
				115					120					125		
CAT	TCC	GCT	AAA	CGG	TGT	GGA	TGT	ATC	TGA	CCCTGGCTCC						718
His	Ser	Ala	Lys	Arg	Cys	Gly	Cys	Ile								
				130												
AGAGACTGCT	GTGTATTGCA	TTCCTGCTAC	ACTGCGAAGA	AAGGGACCAA												768
GGTTCCCAGG	AAATATTTGC	CCAGAAAGGA	AGATAAGGAC	CAAGAAGGCA												818
GAGGCAGAGG	CGGAAGAAGA	AGAAGAAAAG	AAGGACGAAG	GCAGCCATCT												868
GTGGGAGCCT	GTAGAAGGAG	GCCCAGCTAC	AG													900

- (2) INFORMATION FOR SEQ ID NO:4
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 134 amino acid residues

- (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (A) NAME/KEY: inferred amino acid sequence for mature rat GDNF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser	Pro	Asp	Lys	Gln	Ala	Ala	Ala	Leu	Pro	Arg	Arg	Glu	Arg	Asn	Arg	
1				5				10						15		
Gln	Ala	Ala	Ala	Ala	Ser	Pro	Glu	Asn	Ser	Arg	Gly	Lys	Gly	Arg	Arg	
			20					25					30			
Gly	Gln	Arg	Gly	Lys	Asn	Arg	Gly	Cys	Val	Leu	Thr	Ala	Ile	His	Leu	
			35				40					45				
Asn	Val	Thr	Asp	Leu	Gly	Leu	Gly	Tyr	Glu	Thr	Lys	Glu	Glu	Leu	Ile	
	50					55					60					
Phe	Arg	Tyr	Cys	Ser	Gly	Ser	Cys	Glu	Ala	Ala	Glu	Thr	Met	Tyr	Asp	
65					70					75					80	
Lys	Ile	Leu	Lys	Asn	Leu	Ser	Arg	Ser	Arg	Arg	Leu	Thr	Ser	Asp	Lys	
				85					90					95		
Val	Gly	Gln	Ala	Cys	Cys	Arg	Pro	Val	Ala	Phe	Asp	Asp	Asp	Leu	Ser	
			100					105					110			
Phe	Leu	Asp	Asp	Ser	Leu	Val	Tyr	His	Ile	Leu	Arg	Lys	His	Ser	Ala	
	115						120					125				
Lys	Arg	Cys	Gly	Cys	Ile											
130																

(2) INFORMATION FOR SEQ ID NO:5

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 562 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ix) FEATURE:
 (A) NAME/KEY: nucleic acid sequence for human GDNF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATTTTCTCTT	TTCTTTTGA	ACAGCA	AAT	ATG	CCA	GAG	GAT	TAT	CCT	47
			Asn	Met	Pro	Glu	Asp	Tyr	Pro	
			-25						-20	
GAT	CAG	TTC	GAT	GAT	GTC	ATG	GAT	TTT	ATT	89
Asp	Gln	Phe	Asp	Asp	Val	Met	Asp	Phe	Ile	
			-15						-10	

AAA AGA CTG AAA AGG TCA CCA GAT AAA CAA ATG GCA GTG CTT	131
Lys Arg Leu Lys Arg Ser Pro Asp Lys Gln Met Ala Val Leu	
-5 1 5	
CCT AGA AGA GAG CGG AAT CGG CAG GCT GCA GCT GCC AAC CCA	173
Pro Arg Arg Glu Arg Asn Arg Gln Ala Ala Ala Asn Pro	
10 15 20	
GAG AAT TCC AGA GGA AAA GGT CGG AGA GGC CAG AGG GGC AAA	215
Glu Asn Ser Arg Gly Lys Gly Arg Arg Gly Gln Arg Gly Lys	
25 30 35	
AAC CGG GGT TGT GTC TTA ACT GCA ATA CAT TTA AAT GTC ACT	257
Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu Asn Val Thr	
40 45 50	
GAC TTG GGT CTG GGC TAT GAA ACC AAG GAG GAA CTG ATT TTT	299
Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile Phe	
55 60 65	
AGG TAC TGC AGC GGC TCT TGC GAT GCA GCT GAG ACA ACG TAC	341
Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr	
70 75	
GAC AAA ATA TTG AAA AAC TTA TCC AGA AAT AGA AGG CTG GTG	383
Asp Lys Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val	
80 85 90	
ACT GAC AAA GTA GGG CAG GCA TGT TGC AGA CCC ATC GCC TTT	425
Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe	
95 100 105	
GAT GAT GAC CTG TCG TTT TTA GAT GAT AAC CTG GTT TAC CAT	467
Asp Asp Asp Leu Ser Phe Leu Asp Asp Asn Leu Val Tyr His	
110 115 120	
ATT CTA AGA AAG CAT TCC GCT AAA AGG TGT GGA TGT ATC TGA	509
Ile Leu Arg Lys His Ser Ala Lys Arg Cys Gly Cys Ile	
125 130	
CTCCGGCTCC AGAGACTGCT GTGTATTGCA TTCCTGCTAC AGTGCAAAGA	559
AAG	562

(2) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acid residues
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: inferred amino acid sequence for mature human GDNF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly Ala Ala Ala Gly
-5

CGG GAC TTT AAG ATG AAG TTA TGG GAT GTC GTG GCT GTC TGC 88
Arg Asp Phe Lys Met Lys Leu Trp Asp Val Val Ala Val Cys
1 5 10

CTG GTG CTG CTC CAC ACC GCG TCC GCC TTC CCG CTG CCC GCC 130
Leu Val Leu Leu His Thr Ala Ser Ala Phe Pro Leu Pro Ala
15 20

GGT AAG AGG CCT CCC GAG GCG CCC GCC GAA GAC CGC TCC CTC 172
Gly Lys Arg Pro Pro Glu Ala Pro Ala Glu Asp Arg Ser Leu
25 30 35

GGC CGC CGC CGC GCG CCC TTC GCG CTG AGC AGT GAC TGTAAGAACC 218
Gly Arg Arg Arg Ala Pro Phe Ala Leu Ser Ser Asp
40 45 50

GTTCC 223

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: linker

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCCGAATTCG GG 12

(2) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acid residues
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Pro Asp Lys Gln Ala Ala Ala
5

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: nucleic acid sequence from pBluescript SK-76.1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAGAGGAACC GGCAAGCTGC WGMWGYMWGM CCW

33

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acid residues
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Arg Asn Arg Gln Ala Ala Ala Ala Ser Pro
 5 10

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: oligonucleotide DHD-26
- (D) OTHER INFORMATION: N at positions 9 and 12 are inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ARRTTYTTNA RNATYTTTRTC

20

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acid residues
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Lys Ile Leu Lys Asn Leu
 5

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: oligonucleotide primer PD1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GACGGGACTC TAAGATG

17

- (2) INFORMATION FOR SEQ ID NO:16
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer DHD23
 (D) OTHER INFORMATION: N at positions 3, 6, and 18 is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCNGCNGCYT GYTTRTCNGG

20

- (2) INFORMATION FOR SEQ ID NO:17
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer LF2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGAGACAATG TACGACA

17

- (2) INFORMATION FOR SEQ ID NO:18
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer PD2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTGGAGCC AGGGTCA

17

- (2) INFORMATION FOR SEQ ID NO:19
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer PD1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CCCGAATTCG ACGGGACTCT AAGATG

26

- (2) INFORMATION FOR SEQ ID NO:20
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer LFA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGGTGGCCAG AGGGAGTGGT CTTC

24

- (2) INFORMATION FOR SEQ ID NO:21
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer PD3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGCGGATCCA ATAAGGAGGA AAAAAAATGT CACCAGATAA ACAAAT

46

- (2) INFORMATION FOR SEQ ID NO:22
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: oligonucleotide primer PD4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGCGGTACCC AGTCTCTGGA GCCGGA

26

- (2) INFORMATION FOR SEQ ID NO:23
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
(ix) FEATURE:
 (A) NAME/KEY: adapter fragment for plasmid pCJ1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATCTAGAAT TGTCATGTTT GACAGCTTAT CAT

33

- (2) INFORMATION FOR SEQ ID NO:24
(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ix) FEATURE:
 - (A) NAME/KEY: polylinker sequence for plasmid pCJX1-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24

AATTCCCGGG TACCAGATCT GAGCTCACTA GTCTGCA

37